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Evaluation of dentin remineralization after application of bioactive glass 45S5, nano-tricalcium phosphate, and Biodentine

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Abstract

Objective: Dental caries is one of the causes of tooth loss, but it could be stopped by boosting the remineralization process. Bioactive glass 45S5, nano-tricalcium phosphate (nano-TCP), and Biodentine are mineralizing materials. This study aims to evaluate dentin remineralization after bioactive glass 45S5, nano-tricalcium phosphate, and Biodentine are applied.
Methods: Twenty-one dentinal disks (1×1×1 mm) were prepared from 21 extracted premolar teeth. SEM, and XRD tests were performed on dentin samples. The disks were kept in the demineralizing solution for five hours, and the aforementioned tests were carried out again. Then, the disks were divided into three groups (n = 7). The disks received bioactive glass 45S5, nano-TCP, or Biodentine and were covered with a photo-curable glass ionomer. The tests were performed again after 21 days.
Results: Bioactive glass 45S5 and nano-TCP remineralized the dentinal disks, but Biodentine could not.
Conclusion: Bioactive glass and nano-TCP are potentially promising materials for remineralization of tooth structure. It may aid in remineralizing the adjacent demineralized dentin, thus preventing further destruction of the tooth.

Keywords: bioactive glass, dentin, tooth remineralization

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INTRODUCTION

Dental caries is one of the factors that contributes to tooth loss. Despite a multitude of studies on dental caries and preventive methods, caries is still a prevailing problem (Mehta et al. 2014).

Nowadays, dental caries is known as a dynamic and reversible process. Many etiological factors are involved in this process. Imbalance between these factors leads to dental caries (Pradeep and Rao 2011). Caries would come to a halt if there is a balance between demineralization and remineralization processes (Madan et al. 2011).

The preservation of pulp vitality resulting from restorative treatment is crucially important. Capping materials can preserve the vitality, function, and biological activity of the pulp (Aeinehchi et al. 2003). Pulp capping materials act by two different mechanisms: (a) the formation of dentinal bridge due to odontoblastic irritation, like calcium hydroxide and mineral trioxide aggregate (MTA) and (b) the deposition of minerals on the dentin, such as bioactive glass, Biodentine, and tricalcium phosphate (TCP) (Asgary et al. 2008).

Bioactive glass is a mixture of silicon oxide, phosphorus, calcium, and sodium. When bioactive glass is mixed with saliva, calcium and phosphorus ions could be released from its silicated core and form a crystalline layer of hydroxyapatite. This layer remineralizes the tissue and reduces dentinal sensitivity. It has been reported that bioactive glass is effective in reducing sensitivity (Bertassoni et al. 2011, Liu et al., Madan et al. 2011, Reynolds 2008). It is used in bone regeneration, tissue engineering, and tooth remineralization (Wang et al. 2011).

Another material which can be used for remineralization purposes is nano-TCP. This substance with the chemical structure of $Ca_3(PO_4)_2$ is formed through the chemical reactions between oxides and

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hydroxides (like CaO and H_3PO_4) (Dorozhkin 2013). The calcium ions released from TCP can contribute to bone and dentin formation (Jarcho 1981).

Biodentine is tissue compatible and is composed of tricalcium silicate, dicalcium silicate, calcium carbonate, zirconium oxide, and metallic oxide fillers. Biodentine is a good substitute for materials which have a base of calcium hydroxide. Compressive strength is high in Biodentine (Grech et al. 2013, Khedmat et al. 2014, Zanini et al. 2012).

Thus, regarding the importance of the remineralization of the dentin adjacent to the pulpal tissue, this study compared the remineralization potential of bioactive glass, nano-TCP, and Biodentine.

MATERIALS AND METHODS

A number of human premolars extracted for periodontal problems or orthodontic treatments were used in the present study. The teeth with enamel crack, caries, and occlusal and servical wear were excluded from the study. The teeth were measured buccolingually and mesiodistally with a caliper in order to select the teeth which had approximately the same dimensions. Furthermore, the teeth were evaluated for pulp dimension via periapical radiography to ensure maximum similarity of the samples. These measures resulted in the final selection of 21 premolar teeth.

The rationale behind securing dimension uniformity is that the thickness and direction of the dentin tubules will differ according to the changes in tooth dimension and in the distance between the tubules and the dental pulp (Heymann et al. 2013).

Mechanical debridement was performed on the teeth with a scaling instrument (Hu-Friedy, USA). The teeth were kept in thymol 1% in a refrigerator at 4°C for about a month.

The crowns of the teeth were cut off at cemento-enamel junction using a diamond disk on a high-speed rotary handpiece equipped with air and water coolant. The crowns were mounted in an acrylic resin in a cylindrical mold (1.5×4 cm) parallel to one another.

The prepared tooth crowns were cut into dentinal disks (1×1×1mm) using a cutting machine equipped with water coolant and lubricant (Mecatome T201, Presi, France) at a speed of 300 rpm. The cuts were made 0.5 mm above the pulp chamber after taking periapical radiographs and measuring through the use of the Williams probe. The disks were inspected with a stereomicroscope (Catron optical industries, Thailand) at a magnification of 20× to detect any crack or caries. The samples were randomly divided into three groups (n = 7).

Diffraction patterns of the specimens were collected on a Philips X-ray diffractometer (XRD) (Philips, X'pert, Netherlands) with copper target in the 2θ range of 2° to 70°, step size of 0.02°, and λ = 1.54056Å operating at the voltage of 40 kV and current of 30 mA at the rate of 2°/min.

The surface morphology of the specimens was determined by scanning electron microscopy (SEM) (VEGA\\TESCAN, Czech Republic).

SEM, and XRD were performed on all the specimens as positive control. For SEM analysis, the samples were first rinsed with distilled water and were subsequently allowed to dry in an incubator at 37°C for two weeks (INCUCELL, Einrichtungen MMM GMB, Germany). Then, they were gold coated and subjected to SEM. For XRD analysis, the specimens were rinsed and dried with air flow prior to the tests.

Having characterized the specimens, they were demineralized using the 2.2 mM KH_2PO_4, 50mM acetic acid, 2.2 mM $CaCl_2$ solution at pH = 4 for five hours. Following demineralization, the tests were repeated to obtain negative control. Finally, bioactive glass (SiO_2, CaO, P_2O_5), nano-TCP ($Ca_3(PO_4)_2$), and Biodentine ($3CaO.SiO_2$, $2CaO.SiO_2$, $CaCO_3$, ZrO_2, Fe_2O_3, and oxide filler, Septodont (France)) were applied on the disk specimens. 0.5ml of distilled water mixed with powder to have creamy mixture which then was applied on 1mm of dentin surface with applicator. After setting, the mixture and surrounding dentin covered with photo-curable Glass Ionomer (Fuji Ⅱ LC), to evaluate dentin remineralization after application of these three materials. After 21 days the specimens were analyzed with XRD, and SEM.

RESULTS

The following is the results of the characterization tests performed on the samples. It is apparent from the results that while bioactive glass 45S5 and nano-TCP remineralized the dentinal disks, Biodentine failed to do so.

XRD Results

The 2θ between 20° and 35° in **Fig. 1** shows hydroxyapatite crystals with an intensity count of 2107 in sound dentin.

In the pattern of the demineralized dentin (**Fig. 1**), the 2θ between 20° and 35° represents hydroxyapatite crystals with an intensity count of 2566.

In **Fig. 1**, the graph represents $Ca(PO_4)_3$ in nano-TCP.

In addition, the height of the peak at (2θ=31.62) increased for bioactive glass.

The peak at 2θ=31.68 is seen for Biodentine, which is attributed to $CaCO_3$ (Kay et al. 1964, Posner et al. 1958).

SEM Results

Fig. 2a shows sound dentin SEM and EDX micrographs, **Fig. 2b** shows the SEM and EDX micrographs of demineralized dentin, **Fig. 2c** illustrates SEM and EDX micrographs of Biodentine treated dentin,

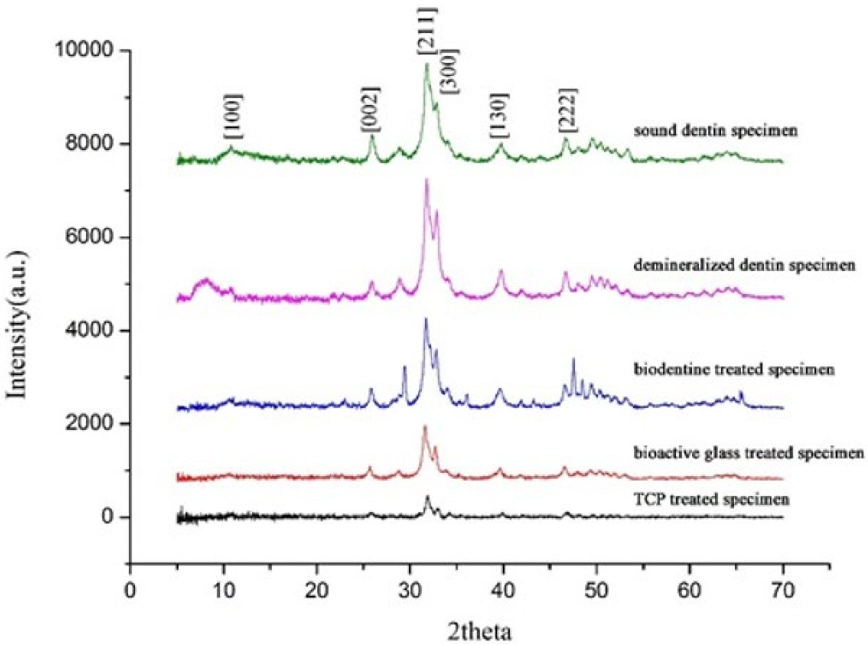

Fig. 1. XRD patterns of the specimens

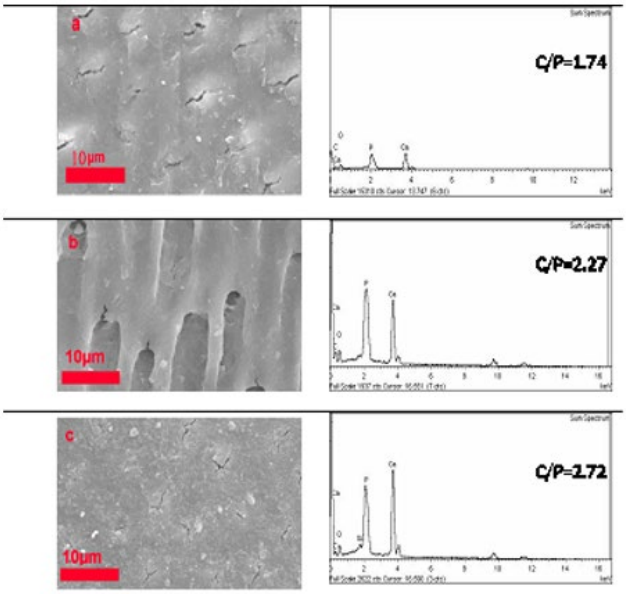

Fig. 2. SEM micrographs at 5000× magnification (a=sound dentin, b=demineralized dentin, c=Biodentine treated dentin)

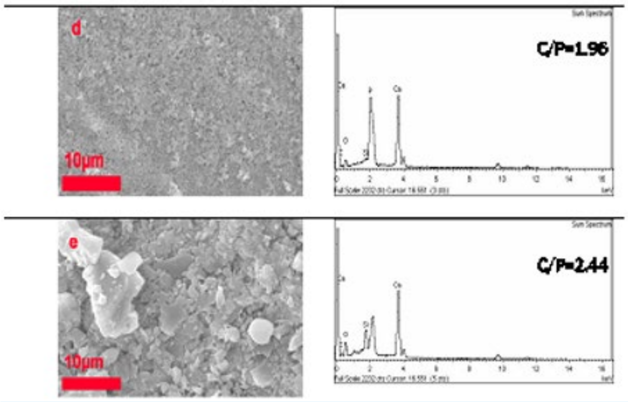

Fig. 2 (continued). SEM micrographs at 5000× magnification (d=nano-TCP treated dentin, e=Bioactive glass 45S5 treated dentin)

Fig. 2d represents the SEM and EDX micrographs of nano-TCP treated dentin and **Fig. 2e** shows Bioactive glass 45S5 treated dentine SEM and EDX micrographs.

DISCUSSION

Strategies to achieve dentin remineralization is at present an important target of restorative dentistry. This is achieved when there is adequate apatite formation which most importantly translates into improved mechanical properties of dentin as a result of intrafibrillar mineralization (Saffarpour et al. 2017).

Bioactive glass may react with saliva and may induce the dissolution of Ca^{2+}, PO_4^{3-} and Si^{4+} ions from the glass surface, resulting in subsequent precipitation of a polycondensed silica-rich layer (Si-gel), which acts as a matrix for the formation of calcium phosphate (CaP), subsequently crystallizing into hydroxycarbonate apatite (HCA) (Andersson and Kangasniemi 1991).

In this study bioactive glass, was applied on demineralized dentin, and after 21 days, the specimens were analyzed using SEM and XRD. XRD analysis revealed the presence of mineral components on dentin surface. The SEM micrographs showed that the entire surface of the dentinal disks was covered with particles of bioactive glass (**Fig. 2e**). The results suggested that incorporated bioactive glass could totally remineralize adjacent demineralized dentin, confirming the hypothesis of this study. Zhong et al. (2015) studied the effect of bioactive glass on dentinal tubule occlusion and used SEM tests to assess tubular occlusion after application of bioactive glass toothpaste, Novamine (desensitizer) toothpaste, and toothpaste with neither bioactive glass nor Novamine. The authors reported that bioactive glass had the best tubular occlusion.

Farooq et al. (2015) studied the dentin tubule occlusion and remineralization competence using SEM, test and reported that the samples were occluded with bioactive glass.

TCP was also used in this study. TCP is a source of calcium and phosphate that are seen in many dental materials such as toothpastes and rinses. TCP contains calcium oxides, calcium phosphates, and free phosphate groups. The dissolution of TCP at oral pH levels releases moderately greater amounts of calcium and phosphate ions than dicalcium phosphate (Vogel et al. 2000).

The SEM results (**Fig. 2d**) obtained here are similar to those reported by Kamath et al. (2017) which demonstrated; TCP plugged the porous defect with resultant decrease in the cavities and micropores and re-establishment of surface integrity in enamel white spot.

Biodentine is a new bioactive cement that is similar to the widely used MTA. It has dentin-like mechanical properties, which may be considered a suitable material for clinical indications of dentin-pulp complex regeneration such as direct pulp capping (DPC) (Halpern et al. 2002). Regarding Biodentine, the SEM images (**Fig. 2c**) reveals that this material did not cover the dentinal disks except in the form of very small particles. The XRD data (**Fig. 1**) also confirms the SEM results.

Dong et al. (2011) applied different concentrations of Biodentine (0, 0.8, 1.2, 1.5 $ml.g^{-1}$) and observed more deposition in 1.2 and 1.5 $ml.g^{-1}$ concentrations of mineral content. With an increase in water content of the Biodentine paste, more deposition was observed. This finding can explain why the present study showed lower Biodentine deposition as we used very low water content (0.5 $ml.g^{-1}$). The lower deposition of Biodentine in the present study might be due to the lower water content of the used paste.

Xie et al. (2016) and Opal et al. (2017) performed a study in order to determine if calcium hydroxide (as common material for pulp capping) can occlude dentinal tubules, but they could not observe such an effect. So this study suggests use of Bioactive glass and nano-TCP for dentin remineralization.

CONCLUSION

The aim of this study is evaluation of dentin remineralization after application of bioactive glass 45S5, nano-tricalcium phosphate, and Biodentine. Bioactive glass 45S5 and nano-TCP remineralized the dentinal disks, but Biodentine could not. Therefore, it can be concluded that bioactive glass and nano-TCP are promising substitutes for pulp capping instead of calcium hydroxide and MTA.

ETHICAL STATEMENT

This study approved by ethical committee of Qazvin University of Medical Sciences with ethical number of IR.QUMS.REC.1396.190. There is no conflict with ethical considerations.

CLINICAL SIGNIFICANCE

This study was *in vitro*, and application of these materials in clinical assessments, can induce some changes in the results.

REFERENCES

Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS (2003) Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. Int Endod J., 36: 225-31. https://doi.org/10.1046/j.1365-2591.2003.00652.x

Andersson OH, Kangasniemi I (1991) Calcium phosphate formation at the surface of bioactive glass in vitro. J Biomed Mater Res., 25: 1019-30. https://doi.org/10.1002/jbm.820250808

Asgary S, Eghbal MJ, Parirokh M, et al. (2008) A comparative study of histologic response to different pulp capping materials and a novel endodontic cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod., 106: 609-14. https://doi.org/10.1016/j.tripleo.2008.06.006

Bertassoni LE, Habelitz S, Marshall SJ, Marshall GW (2011) Mechanical recovery of dentin following remineralization in vitro--an indentation study. J Biomech., 44: 176-81. https://doi.org/10.1016/j.jbiomech.2010.09.005

Dong Z, Chang J, Deng Y, Joiner A (2011) Tricalcium silicate induced mineralization for occlusion of dentinal tubules. Aust Dent J., 56: 175-80. https://doi.org/10.1111/j.1834-7819.2011.01321.x

Dorozhkin SV (2013) Self-setting calcium orthophosphate formulations. J Funct Biomater, 4: 209-311. https://doi.org/10.3390/jfb4040209

Farooq I, Moheet IA, AlShwaimi E (2015) In vitro dentin tubule occlusion and remineralization competence of various toothpastes. Arch Oral Biol., 60: 1246-53. https://doi.org/10.1016/j.archoralbio.2015.05.012

Grech L, Mallia B, Camilleri J (2013) Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. Dent Mater, 29: e20-8. https://doi.org/10.1016/j.dental.2012.11.007

Halpern SD, Karlawish JH, Berlin JA (2002) The continuing unethical conduct of underpowered clinical trials. JAMA, 288: 358-62. https://doi.org/10.1001/jama.288.3.358

Heymann H, Swift EJ, Ritter AV, Sturdevant CM (2013) Sturdevant's art and science of operative dentistry. 6th ed. St. Louis, Mo.: Elsevier/Mosby.

Jarcho M (1981) Calcium phosphate ceramics as hard tissue prosthetics. Clin Orthop Relat Res., (157): 259-78. https://doi.org/10.1097/00003086-198106000-00037

Kamath P, Nayak R, Kamath SU, Pai D (2017) A comparative evaluation of the remineralization potential of three commercially available remineralizing agents on white spot lesions in primary teeth: An in vitro study. J Indian Soc Pedod Prev Dent., 35: 229-37. https://doi.org/10.4103/JISPPD.JISPPD_242_16

Kay MI, Young RA, Posner AS (1964) Crystal structure of hydroxyapatite. Nature, 204: 1050-2. https://doi.org/10.1038/2041050a0

Khedmat S, Dehghan S, Hadjati J, et al. (2014) In vitro cytotoxicity of four calcium silicate-based endodontic cements on human monocytes, a colorimetric MTT assay. Restor Dent Endod., 39: 149-54. https://doi.org/10.5395/rde.2014.39.3.149

Liu Y, Kim YK, Dai L, et al. (2011) Hierarchical and non-hierarchical mineralisation of collagen. Biomaterials, 32: 1291-300. https://doi.org/10.1016/j.biomaterials.2010.10.018

Madan N, Madan N, Sharma V, et al. (2011) Tooth remineralization using bio-active glass - A novel approach. J Adv Oral Res., 2: 45-50. https://doi.org/10.1177/2229411220110209

Mehta AB, Kumari V, Jose R, Izadikhah V (2014) Remineralization potential of bioactive glass and casein phosphopeptide-amorphous calcium phosphate on initial carious lesion: An in-vitro pH-cycling study. J Conserv Dent., 17: 3-7. https://doi.org/10.4103/0972-0707.124085

Opal S, Garg S, Sharma D, et al. (2017) In Vivo Effect of Calcium Hydroxide and Resin-modified Glass Ionomer Cement on Carious Dentin in Young Permanent Molars: An Ultrastructural and Macroscopic Study. Pediatr Dent., 39: 1-8.

Posner AS, Perloff A, Diorio AF (1958) Refinement of the hydroxyapatite structure. Acta Cryst., 11: 308-9. https://doi.org/10.1107/S0365110X58000815

Pradeep K, Rao PK (2011) Remineralizing agents in the non-invasive treatment of early carious lesions. Int Dent J Case Rep., 1: 73-84.

Reynolds EC (2008) Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J., 53: 268-73. https://doi.org/10.1111/j.1834-7819.2008.00061.x

Saffarpour M, Mohammadi M, Tahriri M, Zakerzadeh A (2017) Efficacy of modified Bioactive Glass for Dentin Remineralization and Obstruction of Dentinal Tubules. J Dent (Tehran), 14: 212-22.

Vogel GL, Zhang Z, Carey CM, et al. (2000) Composition of plaque and saliva following use of an alpha-tricalcium-phosphate-containing chewing gum and a subsequent sucrose challenge. J Dent Res., 79: 58-62. https://doi.org/10.1177/00220345000790010901

Wang Z, Jiang T, Sauro S, et al. (2011) Dentine remineralization induced by two bioactive glasses developed for air abrasion purposes. J Dent., 39: 746-56. https://doi.org/10.1016/j.jdent.2011.08.006

Xie F, Wei X, Li Q, Zhou T (2016) In vivo analyses of the effects of polyamidoamine dendrimer on dentin biomineralization and dentinal tubules occlusion. Dent Mater J., 35: 104-11. https://doi.org/10.4012/dmj.2015-209

Zanini M, Sautier JM, Berdal A, Simon S (2012) Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization. J Endod., 38: 1220-6. https://doi.org/10.1016/j.joen.2012.04.018

Zhong Y, Liu J, Li X, et al. (2015) Effect of a novel bioactive glass-ceramic on dentinal tubule occlusion: an in vitro study. Aust Dent J., 60: 96-103. https://doi.org/10.1111/adj.12241